Accurate, strong, and stable reporting of choroid plexus epithelial cells in transgenic mice using a human transthyretin BAC.

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Public Summary:
BACKGROUND: Choroid plexus epithelial cells express high levels of transthyretin, produce cerebrospinal fluid and many of its proteins, and make up the blood-cerebrospinal fluid barrier. Choroid plexus epithelial cells are vital to brain health and may be involved in neurological diseases. Transgenic mice containing fluorescent and luminescent reporters of these cells would facilitate their study in health and disease, but prior transgenic reporters lost expression over the early postnatal period. METHODS: Human bacterial artificial chromosomes in which the transthyretin coding sequence was replaced with DNA for tdTomato or luciferase 2 were used in pronuclear injections to produce transgenic mice. These mice were characterized by visualizing red fluorescence, immunostaining, real-time reverse transcription polymerase chain reaction, and luciferase enzyme assay. RESULTS: Reporters were faithfully expressed in cells that express transthyretin constitutively, including choroid plexus epithelial cells, retinal pigment epithelium, pancreatic islets, and liver. Expression of tdTomato in choroid plexus began at the appropriate embryonic age, being detectable by E11.5. Relative levels of tdTomato transcript in the liver and choroid plexus paralleled relative levels of transcripts for transthyretin. Expression remained robust over the first postnatal year, although choroid plexus transcripts of tdTomato declined slightly with age whereas transthyretin remained constant. TdTomato expression patterns were consistent across three founder lines, displayed no sex differences, and were stable across several generations. Two of the tdTomato lines were bred to homozygosity, and homozygous mice are healthy and fertile. The usefulness of tdTomato reporters in visualizing and analyzing live Transwell cultures was demonstrated. Luciferase activity was very high in homogenates of choroid plexus and continued to be expressed through adulthood. Luciferase also was detectable in eye and pancreas. CONCLUSIONS: Transgenic mice bearing fluorescent and luminescent reporters of transthyretin should prove useful for tracking transplanted choroid plexus epithelial cells, for purifying the cells, and for reporting their derivation from stem cells. They also should prove useful for studying transthyretin synthesis by other cell types, as transthyretin has been implicated in many functions and conditions, including clearance of beta-amyloid peptides associated with Alzheimer's disease, heat shock in neurons, processing of neuropeptides, nerve regeneration, astrocyte metabolism, and transthyretin amyloidosis.

Scientific Abstract:
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